IN OVO GENTAMICIN AND MUCOSAL STARTER CULTURE TO CONTROL *SALMONELLA* IN BROILER PRODUCTION

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Primary Audience: Plant Managers, Hatchery Supervisors, Researchers, Veterinarians

SUMMARY

***Salmonella* continues to be the primary bacterial food safety focus of the U.S. poultry industry. HACCP regulations allow no more than 23% of processed broilers to be positive for *Salmonella*, and this level may be reduced in the future. To be consistently below these levels of *Salmonella*, it is important to control the introduction and proliferation of *Salmonella* on the farm. One of the most effective methods for controlling *Salmonella* is to treat young chicks with competitive exclusion (CE) cultures. The literature suggests that in ovo administration of gentamicin and other antibiotics may reduce the effectiveness of CE. The current study demonstrates conclusively that gentamicin, at a commercial rate of 0.4 mg per egg administered in ovo on Day 18, had no adverse effect on the CE product MSC® (Mucosal Starter Culture). There also appeared to be a cumulative beneficial effect of the gentamicin and the MSC on reduction of *Salmonella*, which enters the chick on the day of hatch.**

Key words: Broilers, competitive exclusion, gentamicin, in ovo, Salmonella
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DESCRIPTION OF PROBLEM

Nurmi and Rantala [1] first used natural intestinal microflora to competitively exclude *Salmonella* from the intestinal tract of broiler chickens. In the ensuing years, there has been a tremendous interest in both research and commercialization of competitive exclusion (CE) products. Not fully characterized (undefined) products, including Broilact® [2], Aviguard® [3], and Mucosal Starter Culture (MSC®; [4]), have been approved and are being used in many regions such as Europe, South America, Japan, and China. In the U.S., the only approved CE product at this time is the fully characterized (defined) product Preempt® [5]. In the commercial field trials of Blankenship et al. [6], MSC® dramatically reduced *Salmonella* colonization of treated chickens to 10% as compared with 41% in untreated control chickens.

Embrex commercialized a technology they had licensed from USDA that is based on the in ovo injection of vaccines and other agents to prevent disease in poultry. This automated

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system punches a small hole through the egg shell and into the air cell of the egg. A needle then delivers the therapeutic product to the embryo through the hole. This usually occurs on Day 18 of the bird's 21-d incubation period. Since it was commercialized in 1993, Embrex Inovoject® [7] has become widely accepted and is now the standard delivery method for Marek's disease vaccine in the U.S. Currently, more than 80% (personal communication) of the U.S. industry uses Inovoject®. To prevent cross-contamination between eggs, Embrex uses an injection of antibiotics in combination with the vaccine. One of the primary antibiotics used for this purpose is gentamicin.

For at least the last 15 yr, there has been concern that antibiotics could adversely affect the ability of CE products to prevent *Salmonella* colonization in young chickens. Bailey et al. [8] found that the antimicrobials in most commercial feed formulations did not affect an undefined CE product, but that high levels of nicarbizin and bacitracin could reduce the effectiveness of the product. Bolder and Palmu [9] found that furazolidone in the feed or trimethoprim-methoxasole sulphate in the drinking water had a short-term affect on Broilact®, but by 4 wk of age, there was still a significant reduction in *Salmonella* compared with control birds. Edens et al. [10] reported that the in ovo administration of gentamicin did not adversely affect the probiotic *Lactobacillus reuteri*. However, it has been recently reported that in ovo or subcutaneous administration of gentamicin sulfate or ceftiofur sodium significantly reduced the establishment of the defined CE PREEMPT™ [11]. Therefore, the objective of this study was to determine whether commercial application of gentamicin in ovo would adversely affect the efficacy of the undefined CE product MSC®.

Materials and Methods

MICROORGANISMS, MAINTENANCE, AND INOCULUM PREPARATION

Salmonella typhimurium 3333/0, a spontaneous nalidixic acid-resistant strain, was obtained from S. E. Craven [12]. The stock culture was maintained at −80°C on latex beads [13] Subcultures were streaked the day before inoculation onto BGS agar [14] plates with 200 ppm nalidixic acid [15]. Plates were incubated at 35°C overnight.

Inoculum was prepared by suspending three isolated colonies in 0.85% sterile saline and adjusting the absorbance to 0.2 at 540 nm. Approximately 10^8 cfu were obtained. Serial 1:10 dilutions were prepared in 9-mL saline blanks, and 0.1 mL of the appropriate dilution was administered by oral gavage into the chicks.

EGGS AND CHICK HANDLING

Three replicate trials were conducted. For each trial, approximately 500 (Day 18) eggs were obtained from local hatcheries. The eggs were transported to the laboratory in insulated containers. At the laboratory, the eggs were candled and surface-decontaminated with 0.5% sodium hypochlorite [17]. Using a Dremel Rototool [18], a small hole was drilled into each air cell. Each egg was injected into the amnion (by deep injection of approximately one inch) with 0.2 mL of either Marek's sterile diluent [19] or Marek's sterile diluent with 2 mg/mL gentamicin [15], yielding a 0.4-mg dose of gentamicin. The eggs were placed (36 per incubator) into incubators [20]. The temperature and relative humidity were maintained at 37.2°C and approximately 75%, respectively.

On the day of hatch, the chicks were removed from the incubators and placed into isolation floor pens at the Poultry Science Department Farm of the University of Georgia (Athens, GA). There was a total of eight treatment groups for each of the three replicate trials. Treatment Groups 1 through 4 were gavaged with approximately 10^4 cfu of a nalidixic acid resistant strain of *Salmonella typhimurium* approximately 1 h prior to MSC® treatment. Group 1 contained only birds from eggs treated with gentamicin. Group 2 contained birds that had not received gentamicin treatment but were gavaged with 0.2 mL of a 10^8 dilution of MSC®. Group 3 contained birds that had no gentamicin or MSC® treatment. Group 4 contained birds that had both the gentamicin treatment and the MSC® treatment. Treatment Groups 5 through 8 were gavaged with approximately 10^6 cfu of a nalidixic acid resistant strain of *Salmonella typhimurium* approximately 24 h after MSC® treatment. Group 5 contained only birds from eggs treated with gentamicin. Group 6 contained birds that

TABLE 1. Recovery of *Salmonella* from chickens when *Salmonella* challenge was before administration of the MSC®,[A]

GENTAMICIN		TRIAL 1		TRIAL 2		TRIAL 3		AVERAGE	
PER EGG	MSC	n	Avg. cfu	n	Avg. cfu	n	Avg. cfu	n	Avg. cfu
0.4 mg	No	20	5.87	20	0.70	20	4.71	60	3.76[abc]
0 mg	Yes	20	3.96	20	5.70	20	4.40	60	4.69[b]
0 mg	No	20	6.25	20	6.36	20	6.33	60	6.31[a]
0.4 mg	Yes	20	0.00	20	0.10	20	0.15	60	0.08[c]

[a–c]Data with no common superscripts are significantly different.
[A]Wayne Farms LLC, Oakwood, GA.

had not received gentamicin treatment but were gavaged with 0.2 mL of a 10^8 dilution of MSC®. Group 7 contained birds that had no gentamicin or MSC® treatment. Group 8 contained birds that had both the gentamicin treatment and the MSC® treatment.

MICROBIOLOGICAL SAMPLING

Ten birds from each unit were sacrificed on Day 7 by cervical dislocation and weighed. The ceca was aseptically removed. The ceca were placed into individual Stomacher 80 bags [14] and weighed; a volume of universal pre-enrichment (UP) [15] broth (Trials 1 and 2) or buffered peptone water (BP) [15] equal to three times the weight of the ceca was added to the bags. The ceca were stomached for 60 s. The samples were plated onto BGS agar [15] with 200 ppm nalidixic acid [16] using the plating method of Bailey et al. [8]. The plates were incubated at 35°C overnight.

Samples negative for *Salmonella* from direct streaks were re-streaked from the overnight enrichment broth and incubated for 24 h at 35°C. Colony-forming units were calculated by adding 1.7 $\log_{10}$ factors to the estimated log counts on the A plate or 3.7 $\log_{10}$ factors to the estimated log counts from the B plate [8]. Re-streaks were assigned a $\log_{10}$ value of 1.5. Average cfu were calculated for each treatment.

Results and Discussion

When *Salmonella* was given before the administration of the MSC® (Table 1) chicks that received only MSC® and no gentamicin had only about a 1.5 log reduction in the level of intestinal *Salmonella*. This observation was consistent with the published literature that shows CE only works if is provided to the chicks before they are exposed to *Salmonella*. However, a very exciting observation was that when the chicks received the gentamicin in ovo, the *Salmonella* immediately after hatch, and then the MSC® 1 d later: there was a statistically significant ($P < 0.05$) cumulative effect and an almost total elimination of *Salmonella* in all three replications.

When the *Salmonella* was given 1 d after administration of MSC® (Table 2), there was a 4 log reduction in MSC®-treated birds compared with untreated control birds. When gentamicin had been given in ovo, the MSC® after hatch,

TABLE 2. Recovery of *Salmonella* from chickens when *Salmonella* challenge was after the administration of the MSC®,[A]

GENTAMICIN		TRIAL 1		TRIAL 2		TRIAL 3		AVERAGE	
PER EGG	MSC	n	Avg. cfu	n	Avg. cfu	n	Avg. cfu	n	Avg. cfu
0.4 mg	No	ND[B]	ND	ND	ND	20	6.08	20	6.08[abc]
0 mg	Yes	20	1.21	20	2.69	20	2.06	60	1.99[b]
0 mg	No	20	5.96	20	6.33	20	5.75	60	6.01[c]
0.4 mg	Yes	20	0.22	20	3.51	20	0.52	60	1.42[b]

[a–c]Data with no common superscripts are significantly different.
[A]Wayne Farms LLC, Oakwood, GA.
[B]No data available.

and the *Salmonella* 1 d later, there was still a 4.5 log reduction in treated chicks compared with control chicks.

The in ovo administration of gentamicin at the commercial application of 0.4 mg per egg did not adversely affect the efficacy of the undefined MSC®. The additive effect of in ovo gentamicin and MSC® after exposure to *Salmonella* is similar to the observation of Edens [10], who reported that the combination of gentamicin and *L. reuteri* had an additive effect of decreasing *E. coli*-associated mortality. These data show that commercial application of gentamicin through Inovoject® will in no way diminish the effectiveness of MSC®.

Conclusions and Applications

1. MSC® CE microflora significantly reduced *Salmonella* colonization of chicks' ceca if the culture was given to the chicks before exposure to *Salmonella*.
2. In ovo administration of gentamicin did not affect the ability of MSC® administered immediately after hatch or 1 d after hatch to reduce *Salmonella* colonization of ceca in young chicks.
3. There appears to be a cumulative effect of in ovo gentamicin and MSC® on the reduction of *Salmonella* that may colonize the ceca of chicks immediately after hatch. This combination treatment could help to reduce *Salmonella* cross-contamination in the hatch cabinet.

References and Notes

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STATISTICAL ANALYSIS

Data were analyzed for significant difference using a dependent *t*-test (Statistica, StatSoft, Inc., Tulsa, Ok). Significance implies $P < 0.05$.